9th Eastern European Young Water Professionals Conference

the international water association

CONFERENCE PROCEEDINGS

Uniting Europe for Clean Water:

Cross-Border Cooperation of Old, New and Candidate Countries of EU, for identifying problems, finding causes and solutions

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Editors:

Maryna Feierabend

Olha Novytska

Vince Bakos

Typesetting:

Maria Danilycheva

Maja Djogo

Éva Zofia Sebestyén

Cover Design:

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Wastewater Treatment of Hospitals from Pharmaceutical and Bacterial Contaminants

I. Yermakovych[*], G. Kochetov[**], N. Samoilenko*** and L.Mårtensson[****]

[*]Department of Occupational Safety and Environment at Kyiv National University of Construction and Architecture, 31,Povitroflotsky Avenue, Kyiv, 03037, Ukraine
(E-mail: *iryna.yermakovych@gmail.com*)
**Department of Chemistry, KyivNationalUniversityofConstructionandArchitecture, 31,Povitroflotsky Avenue, Kyiv, 03037, Ukraine (E-mail: *gkochetov@gmail.com*)
***DepartmentofChemicalEquipmentand IndustrialEcology, National Technical University "Kharkiv Polytechnic Institute", 21, Frunze str., 61002, Kharkiv, Ukraine
(E-mail*: nataliiasamoilenko@gmail.com*)
**** Department of SchoolofEducationandEnvironment, KristianstadUniversity, Elmetorpsvägen 15, SE-291 88 Kristianstad, Sweden (E-mail: *lennart.martensson@hkr.se*)

Abstract
The medical establishments are considered the main source of wastewater containing micropollutants like pharmaceuticals and pathogens. Performing of the local sewage treatment of hospitals from pharmaceuticals and their simultaneous disinfection in the field of direct formation can virtually eliminate inflow into the municipal sewage system (MSS) of this type of polluted wastewaters. In this case, the anodic oxidation method of pharmaceuticals contained in the sewage of hospitals is proposed with using in a treated sewage of 500 mg/l of sodium chloride and RuO_2 -anode. Via the method of liquid chromatography mass spectrometry was confirmed the complete destruction of diclofenac and beta-estradiol molecules in the treated wastewater as well as furosemide, atenolol, cefuroxime and mixtures of all five substances. Microbiological researches of treated water samples showed the full inhibition of the cultivation of pathogenic culture of *Escherichia Coli*.

Keywords
Pharmaceutical substances; natural waters; electrochemical destruction; disinfection; *Escherichia Coli*

Introduction

The problem of entering of the micropollutants into the environment in the form of persistent pharmaceutical substances (PhSb) and bacterial contaminants becomes more urgent. Pharmaceuticals and their derivatives are detected in wastewaters, activated sludge, oceanic and rivers' sediments, in filtrates of municipal dumping in such countries as France, Germany, Great Britain, Denmark, Netherlands, Sweden and USA. Some of their remains were also observed in drinking waters, glacial, underground and oceanic waters. At the same time, the greatest danger is the presence of these compounds in surface waters, where they accumulate and can be involved in the trophic chains (McClellan, 2010; Bendz, 2005; Boxall, 2004). In addition, the negative impact on aquatic ecosystems has inappropriate microorganisms for them that break the structure of the natural biota groups and environmental ecosystem functions.

The main source of wastewater formation containing the combination of pharmaceutical and bacterial contaminations is medical establishments. Their sewage inflows into municipal wastewater treatment plant, where pharmaceuticals present the most persistent organic micropollutants, which are practically not destroyed and dumping into natural waters. Moreover, these kinds of pollutants are contained in household sewage as well as in wastewater, which are formed during the process of destruction of unqualified pharmaceuticals, sludge bank. All PhSb

researched in this study as contaminants have varied chemical structures and belong to the organic compounds having the aromatic groups with developed chains.
Diclofenac refers to the most widely used drugs in hospitals. It belongs to the most frequently found pharmaceuticals in surface waters. Diclofenac has properties of a substance that is difficult to biodegrade, and which cannot be destroyed at the municipal wastewater treatment plants (MWTP) (Sawchuk, 1995). The drug and its metabolites have a highly acute toxicity even at very low concentrations $\leq 5 \cdot 10^{-6}$ g/l. It causes kidney damage, and changes in fish gills (Triebskorn, 2004;Schwaiger, 2004).

Diclofenac is included in the list of priority substances in the field of water policy according to the Proposal for a directive of the European parliament and of the Council (Falås, 2012). Furosemide is a highly active diuretic, which is very often used in cardiology departments of hospitals. Its concentration in surface waters could reach more than $0{,}605 \cdot 10^{-6}$ g/l (Ferrari, 2011). Atenolol is widely used β-blocker in some European countries. It is a primary drug for the treatment of hypertension. Its concentration in surface waters could be more than $0{,}083 \cdot 10^{-6}$ g/l (Alder, 2010). Cefuroxime is the most common group of semisynthetic cephalosporin antibiotic of the second generation used in hospitals for oral or parenteral treatment (Grung, 2008).

A preliminary treatment and disinfection of hospitals wastewaters before their discharging into the sewer system can significantly reduce the flow of pharmaceuticals and bacterial contaminants into treatment facilities and inhabited locality as well as in natural waters. For this purpose, it is suggested to use electrochemical destruction of PhSb contained in hospital wastewaters. Moreover, another positive effect of this method can be wastewater disinfection from the most common bacterial contaminants.

MATERIAL AND METHODS

The research was carried out on the solutions that simulated hospital wastewater of general treatment. For contaminants were used five powdered pharmaceutical substances: diclofenac ([2-(2,6-Dichloroanilino)phenyl]acetic acid), β-estradiol ((8*R*,9*S*,13*S*,14*S*,17*S*)-13-methyl-6,7,8,9,11,12,14,15,16,17-decahydrocyclopenta[*a*]phenanthrene-3,17-diol), furosemide (4-Chloro-2-[(furan-2-ylmethyl)amino]-5-sulfamoylbenzoic acid), atenolol (*RS*)-2-{4-[2-Hydroxy-3-(propan-2-ylamino)propoxy]phenyl}acetamide), cefuroxime ((6*R*,7*R*)-3-{[(aminocarbonyl)oxy]methyl}-7-{[(2*Z*)-2-(2-furyl)-2-(methoxyimino) acetyl]amino}-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid) of firm Sigma-Aldrich (Belgium) (chemical purity is 99,9%). The concentration of the solutions of pharmaceutical substances was limited by the value of 0.375 mg/l.

For increasing electric conductivity and decreasing of electrical energy consumption, into the model of wastewater were added sodium chloride (NaCl) and sodium sulfate (Na_2SO_4) with the concentration of 300-1000 mg/l (brand MerckKGaA (Germany) (chemical purity 99.9%). Chemical solvents (methanol, formic acid,acetonitrile and KH_2PO_4 solution) in ultrapure form intended only for liquid chromatography HPLC Fluka-Company (Germany) were used as the mobile phase. The electrochemical destruction was performed in open cylindrical glass cell. The source of direct current was used the laboratory device DC Power Supply model GPS-3030D.

As electrodes were used a graphite rod cathode and a platinum wire anode (Pt), and the cathode plate high alloy steel and a titanium grid anode coated with ruthenium oxide (RuO_2). The process was carried out with the following parameters: current was 0.19-0.59 A (RuO_2/Pt), voltage - 29.5 - 31.5 B (RuO_2/Pt), temperature of the solution was 25 °C.

For providing mass transfer to the electrodes of the pharmaceutical substance, the model solution is continuously mixed by a lab magnetic stirrer (800 rev/min). The pH of the medium was measured with a pH meter Jenway 3510 pH.

The degree of substance degradation in the test samples was controlled by using the liquid chromatography with ultraviolet detector (LC-UV) Shimadzu and HPLC liquid chromatography and mass spectrometry (LC-MS) type LC-MS Waters QTOF Xevo G2 Waters Acquity UPLC. For this aim, methods of the detection and control of pharmaceutical substances were developed for the model solutions on the LC-UV and LC-MS equipment.

For evaluation of the bactericidal properties for electrochemical treatment of modeling wastewater, as test object, a pathogenic bacterial culture - *Escherichia Coli (E. Coli)* was used, as well as a modified method for determining the dehydrogenase activity (DHA), which is often applied at process control work at aeration tanks of biological treatment plants (Makuch, 2010).

RESULTS AND DISCUSSION

The initial concentration of each PhSb separately and their mixture in model solution was 3 mg/l. Before performing the electrochemical destruction of pharmaceuticals mixture of model wastewater, it was found the optimum parameters of the process for each component separately.

In the research was determined the kinetics of PhSh removal from modeling solutions during destruction process via the changing composition of the electrolyte, its duration and the anode material. It was determined that the sodium sulfate salt solution is ineffective like an electrolyte for the oxidation resistant pharmaceutical molecules. A greater effect of application is provided by the salt solution of sodium chloride with the concentration 500 mg/l. In Table 1 is shown a summary of data about the modes of PhS degradation by using anodic oxidation method.

Table 1. Summarized data of the characteristics of the degradation process of PhS by anodic oxidation method

Name of drug	Electrolyte NaCl, g/l	Current (RuO_2/Pt), A	Voltage (RuO_2/Pt), V	Process time (RuO_2), min	Process time (Pt), min
Diclofenac	0,5	0,54/0,39	29,0-31,5	6	≤60
β-estradiol	0,5	0,59/0,35	29,0-31,5	1	≤6
Furosemide	0,5	0,37/0,28	29,0-31,5	1	1
Atenolol	0,5	0,59/0,28	29,0-31,5	1	1
Cefuroxime	0,5	0,48/0,15	29,0-31,5	1	≤5
Mixture	0,5	0,55 (RuO_2)	29,0-31,5	10	–

The obtained experimental data about degradation of substances demonstrate that the process of PhS destruction performed on RuO_2 anode is more effective than on platinum. Four substances were destroyed after the first minute of the process beginning. Only for the most persistent molecule of anti-inflammatory drug - diclofenac, destruction lasted about 5 minutes.

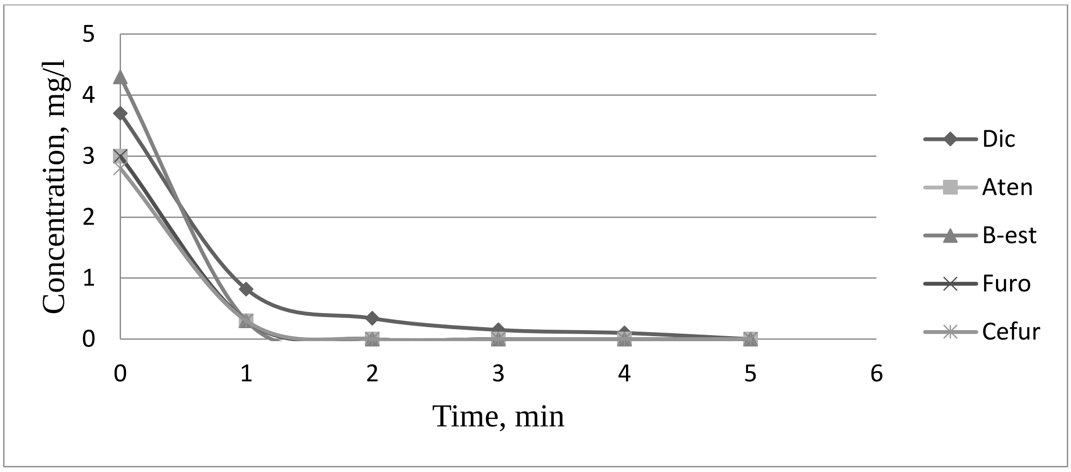

Figure 1. Total kinetic process of PhSb destruction using the RuO2 anode. Concentration of NaCl – 500 mg/l

Application of the platinum as anode for this type of destruction of pharmaceutical molecules was less effective.

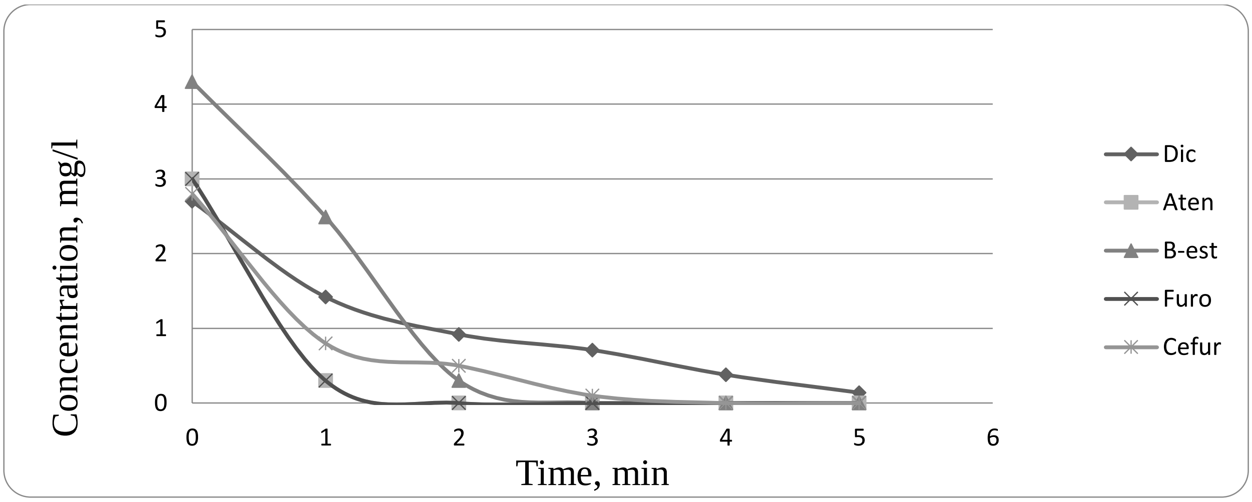

Figure 2. Total kinetic process of PhSb destruction using of the platinum anode. Concentration of NaCl - 500 mg/l

The degree of degradation of each PhSb was analyzed on two types of analytical equipment, namely LC-UV and LC-MS. The mixture of five PhSb was controlled by the LC-MS equipment, as only this analytical equipment allows to perform more efficient separation of complex mixtures.

Figure 3a shows the LC-MS chromatography with UV detector of PhSb mixture before electrochemical destruction. The scanning of the mixture samples on this type of equipment was carried out at 254 nm, which is typical for detection the substances with aromatic functional groups. Chromatogram peaks of listed substances were clearly identified at this wavelength.

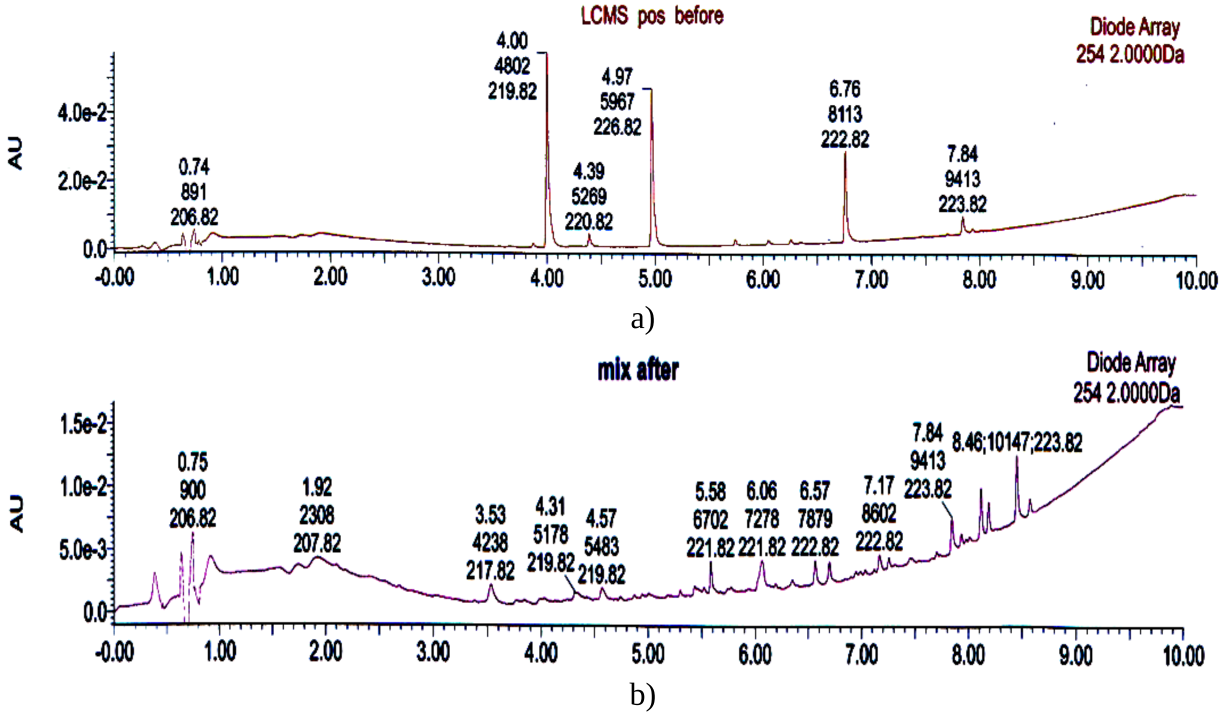

Figure 3. Chromatograms of PhSb mixture LC-MS type with UV detector before the destruction process (a) and after the destruction process (b)

The duration of detection of each PhSb constituted (min): atenolol - 0.78; cefuroxime - 4.02; furosemide - 4.98; beta-estradiol - 5.88; diclofenac - 6.76.
Destruction of these PhSb mixture was carried out for 10 min and then the mixture was analyzed on LC-MC equipment.

Fig. 3 (b) is showed the result of scanning PhSb mixture after the electrochemical oxidation. They were observed that all five of PhSb after electrochemical destruction process were not determined on the chromatogram.

Thus, the analysis of the model solution containing mixture of five PhSb performed on LC-MS equipment has shown that all of these molecules were completely destroyed during the electrochemical destruction. Thereby, the obtained experimental data demonstrate that the method of electrochemical destruction of resistant to biodegradation PhSb is efficient and appropriate for local treatment of wastewater containing such kind of pollution.

Experiments of identification antibacterial properties of the PhSb solutions before and after the electrochemical destruction were performed on biotest culture of *E.Coli* at a density of the culture liquid 10^6 CFU/ml.

Electrochemical treatment of model solutions was performed with the same parameters, which were obtained during the research on the destruction of PhSb. As active substances were used the model solutions of diclofenac, ibuprofen, beta-estradiol before and after the electrochemical treatment. The values of the test culture of DHA under the influence of PhSb solutions are presented in Table 2.

Table 2. Influence of PhSb solutions and their products of the electrochemical destruction on DHA of test culture *E.Coli*

Solutions of PhSb before or after electrochemical treatment	DHA of biotest *E.Coli*, mg formazan (g·biomass·min)$^{-1}$
Diclofenac (3 mg/l), NaCl (500 mg/l)	6,63
Solution of diclofenac after electrochemical destruction	0
Mixture of diclofenac (3 mg/l), ibuprofen (3 mg/l), beta-estradiol (3 mg/l), NaCl (500 mg/l) after destruction process	0
Control (culture liquid of *E.Coli* with out of PhSb)	7,45

According to the Table 3 the pronounced effect of inhibition (11% compared to the control sample), i.e. inhibition of DHA of test culture *E. Coli* have already been registered under the influence of one substance - diclofenac.

In all the studied models of wastewater containing PhSb after the electrochemical destruction were observed complete inhibition DHA test cultures. The high disinfecting effect of the solution after the electrochemical treatment can be explained by the influence of the active ion of chlorine molecules on *E. Coli* – Cl^-, ClO_2, ClO^-_3, $HClO^-$, formed during electrochemical destruction process.

Based on the experimental data was defined relation, which determines the inhibition constant of *E. Coli* metabolism under the influence of diclofenac - active substance concentration at which the metabolic processes activity is reduced by a half (see Fig.5). DHA of *E.Coli* test strain is inhibited by 50% under the influence of diclofenac solution at the concentration of 0.22 mg/mg of biomass, which corresponds to the results of other researchers (Pabitra Basnyat, 2010).

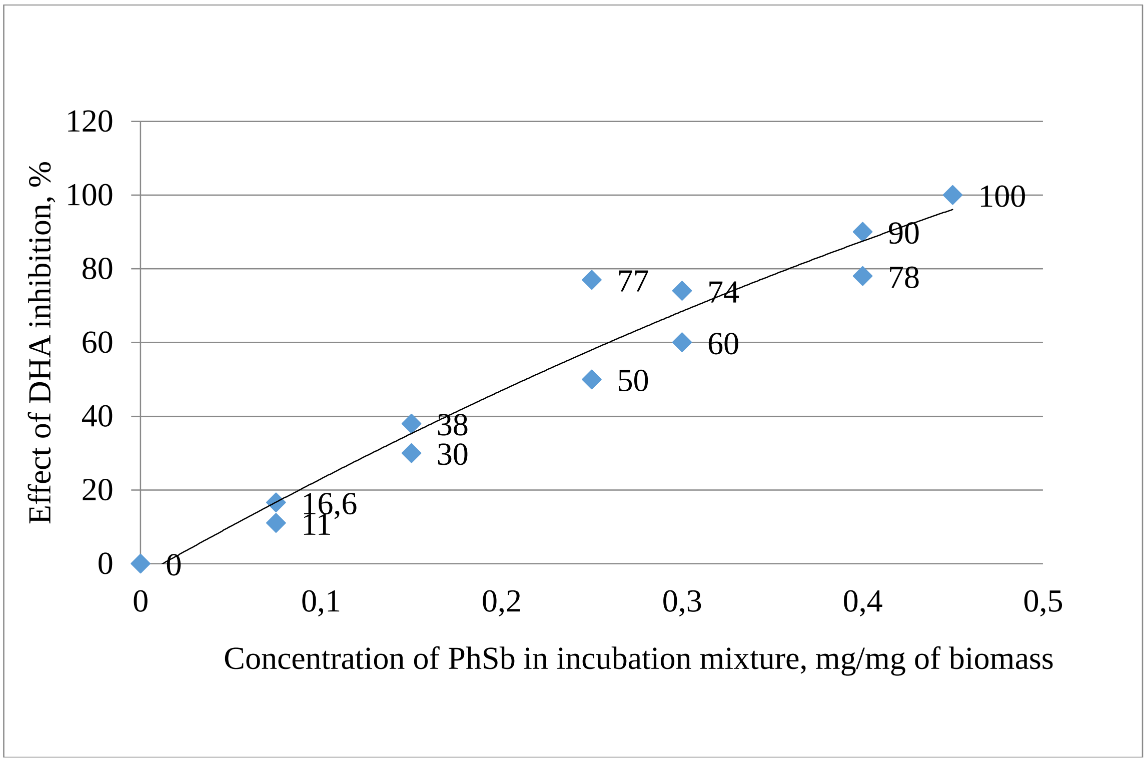

Figure 4. Inhibitory addiction effect from the concentration of diclofenac

Such high concentrations of specific inhibitory substances is not observed either at MSS nor in the natural environment. However, considering the ability of flora and fauna to accumulate of chemical substances, it couldn't be excluded the possibility of a significant accumulation of PhSb in natural waters of econiches. Furthermore, bacteria of *E. Coli* has a very high resistance to chemical agents. (Fotadar, 2005; Small, 1994; Vogt, 2005*)*. For example, the inhibition constant autochthonous microflora, microfauna and flora of natural waters can be significantly lower than 0.22 mg/kg of biomass. It can be noted that the electrochemical treatment of hospital sewage is not only destroy the containing environmentally hazardous PhSb, but inhibits the bacterial flora always presented in such effluents. This increases the reliability of regulatory disinfection measures before its flowing into a common municipal sewerage system.

In order to determine the possibilities of inhibitory effect of wastewater after the electrochemical treatment on the activated sludge of biological treatment plant, it was taken into account the dilution of their flow at the biological treatment plant in Kharkov, Ukraine. In addition, the research of biotesting wastewater was performed under the same composition by determination the DHA of *E.Coli.* The experimental results were shown that the hospital wastewater that has passed pre-cleaning from PhSb by electrochemical treatment at reception on MSS did not have a negative impact on biocenosis of activated sludge of biological treatment.

Thus, application of the electrochemical destruction method of PhSb is an efficient way also for disinfection of hospital sewage from *E.Coli* that increases their sanitary and epidemic safety.

CONCLUSIONS

The local treatment of hospital wastewater from pharmaceutical and bacterial contaminants enables to eliminate these kinds of pollutants to flow into the MSS and wastewater treatment plant of the city that is significantly increasing the environmental safety of natural waters. The application of electrochemical destruction for wastewater treating before discharging into the municipal waters allows removing the harmful for the environment pharmaceutical substances and carrying out their disinfection from bacterial contaminants in a single process.

Experimentally have been determined the optimal regimes and conditions of the process of electrochemical destruction and kinetic indicators of complete destruction of resistant pharmaceutical substances of diclofenac, beta-estradiol, atenolol, furosemide and cefuroxime in concentrations maximally close to the similar in wastewater of hospitals. Maximum efficiency of purification process is achieved by using RuO_2 anode, current of 0.5 A and a voltage of 31.5 V in a medium with concentration of 500 mg/l of sodium chloride.

Experimentally is proved the efficiency of electrochemical destruction of five pharmaceutical substances as well as their mixtures with sodium chloride solution (500 mg/l) using RuO_2 anode during 10 minutes. It is established that during the process of electrochemical oxidation of PhSb at the certain process parameters is proceed the total destruction.

It has been confirmed the possibility during the anodic oxidation process of the simultaneous complete inhibition of pathogenic culture *Escherichia Coli*.

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